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DATE: Tuesday, August 27, 2002 Printable Copy Create Case

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DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=ADJ			
L11	l5 and l9	15	L11
L10	l5 same l9	0	L10
L9	l2 same l7	3689	L9
L8	l7 and l6	1	L8
L7	yeast or saccharomyces	79263	L7
L6	l5 same l3	3	L6
L5	angiotensin	10237	L5
L4	angiotension	417	L4
L3	l1 same l2	119	L3
L2	milk	91073	L2
L1	lactobacillus helveticus	319	L1

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L11: Entry 15 of 15 File: DWPI Jul 30, 1998

DERWENT-ACC-NO: 1994-268691
DERWENT-WEEK: 199835

TITLE: Prepn. of peptide which inhibits angiotensin conversion enzyme - by lactic acid bacterium culture of material with specified tri:peptide sequence

PATENT-ASSIGNEE:

ASSIGNEE	CODE
CALPIS SHOKUHIN KOGYO KK	CALV

PRIORITY-DATA: 1992JP-0298887 (November 9, 1992)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
JP 2782153 B2	July 30, 1998		008	C12P021/02
JP 06197786 A	July 19, 1994		008	C12P021/00

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
JP 2782153B2	November 4, 1993	1993JP-0275790	
JP 2782153B2		JP 6197786	Previous Publ.
JP06197786A	November 4, 1993	1993JP-0275790	

INT-CL (IPC): C07K 3/12; C12P 21/00; C12P 21/02; C12P 39/00; C12P 21/02; C12R 1/225; C12P 21/02; C12R 1/865; C12P 21/00 ; C12R 1/225; C12R 1/865

ABSTRACTED-PUB-NO: JP06197786A
BASIC-ABSTRACT:

To prepare a peptide (I) which inhibits angiotensin conversion enzyme (ACE), a lactic acid bacterium and opt. a yeast are cultured in a medium contg. a peptide (II) and/or a protein (III) and then purified. (I), (II) and (III) Include the sequence Ile-Pro-Pro and/or Val-Pro-Pro.

Pref. purificn. is by centrifugation of the culture and purification of the supernatant.

ADVANTAGE - The method prepares an ACE inhibiting peptide easily at low cost.

In an example, 18 g defatted milk powder in 200 g water was sterilised at 115 deg.C for 20 minutes and cooled to room temp.. 2.0 x 10 power 7/ml of Lactobacillus helveticus JCM-1003 and 2.0 x 10 power 5/ml of Saccharomyces cerevisiae ATCC-9804 were inoculated into it and cultured at 37 deg.C for 24 hrs. to prepare the primary starter. 360 g defatted powder milk in 4 kg water was sterilised at 90 deg.C, cooled, the primary starter was added and it was cultured at 37 deg.C for 24 hrs. to prepare the secondary starter. Sterilised 9.0 kg defatted powder milk in 100 kg water was inoculated with the sec. starter and cultured at 37 deg.C for 24 hrs. to give 112 kg of fermented milk. The content of the peptide (I) in the fermented milk was determined

by HPLC. It contained 3060 mg Val-Pro-Pro and 1960 mg Ile-Pro-Pro. The peptide (I) had an ACE inhibiting activity of 4.5 x 10 power 6 U.

CHOSEN-DRAWING: Dwg.0/2

TITLE-TERMS: PREPARATION PEPTIDE INHIBIT ANGIOTENSIN CONVERT ENZYME LACTIC ACID BACTERIUM CULTURE MATERIAL SPECIFIED TRI PEPTIDE SEQUENCE

DERWENT-CLASS: B04 D16

CPI-CODES: B04-C01; B04-N03B; B14-F02B1; D05-C11;

CHEMICAL-CODES:

Chemical Indexing M2 *01*
Fragmentation Code
F011 F012 F019 F423 F499 H1 H100 H181 H2 H212
J0 J013 J1 J111 J3 J311 J371 M280 M314 M315
M321 M333 M340 M342 M349 M381 M391 M413 M510 M522
M530 M540 M720 M903 M904 N131 N132 N161 N513 P526
P616 Q233 V814 V901 V911
Markush Compounds
199433-17201-P

Chemical Indexing M1 *02*
Fragmentation Code
M423 M720 M903 N131 N132 N161 N513 P526 P616 Q233
V600 V631 V814 V901 V917

SECONDARY-ACC-NO:
CPI Secondary Accession Numbers: C1994-122518

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L8: Entry 1 of 1 File: USPT Jun 16, 1998

DOCUMENT-IDENTIFIER: US 5766940 A
TITLE: Plasmid and plasmid vector

Brief Summary Text (3):
Lactobacillus helveticus has been used for a long time as a typical dairy lactic acid bacteria starter for production of a fermented milk. It is known that one of the properties of Lactobacillus helveticus is strong proteolytic activity (Yamamoto, N., et al., Biosci. Biotech. Biochem., 58, 776-778 (1994)). Also, it has been reported that the fermented milk by Lactobacillus helveticus contains bioactive peptides which exhibit inhibitory effect against angiotensin converting enzyme which plays an important role in elevating blood pressure (Nakamura, Y. et al., J. Dairy Sci., 78, 777-783 (1995)), and the fermented milk by Lactobacillus helveticus has strong hypotensive effect due to these peptides (Nakamura, Y., et al., J. Dairy Sci., 78, 1253-1257 (1995)). As a method for effective utilization of strains having such properties, methods of genetic engineering utilizing genetic recombination techniques have conventionally been known. Although many plasmids for lactic acid bacteria have been reported, there are few reports on plasmids for lactic acid rod bacteria, Lactobacillus helveticus. Thus, there are few reports on host-vector system of Lactobacillus helveticus .

Detailed Description Text (31):
The replication origin region of a microorganism may include, other than the replication origin region of pACYC177, a publicly known replication origin region of e.g. pBR322 and pBR329 derived from Escherichia coli. Further, replication origin regions such as pUB110,1 YEp24, pVA838 derived from Bacillus subtilis or yeast may also be used.

Detailed Description Text (40):
Subsequently, purification of the plasmid was performed according to the following procedure. The collected cells were suspended in 0.2 ml of 25% sucrose and 10 mM tris-HCl buffer (pH 7.0) containing 10 mg/ml of lysozyme manufactured by SIGMA CHEMICAL CO., 1 mg/ml of N-acetylmuramidase manufactured by SEIKAGAKU CORP., and incubated at 37.degree. C. for 30 minutes. The cell suspension was then admixed with 0.4 ml of 3% SDS-0.2N NaOH solution and allowed to stand at room temperature for about 5 minutes. The suspension was then further admixed with 0.3 ml of 3 M sodium acetate (pH 4.8) solution, allowed to stand on ice for 5 minutes, and centrifuged (15,000 rpm, 5 minutes) for collecting the supernatant. The supernatant was admixed with ethanol of twice the volume of the supernatant, and allowed to stand at -80.degree. C. for 20 minutes. Subsequently, a precipitate was collected by centrifugation (15,000 rpm, 15 minutes), washed with 70% ethanol, dried, and then dissolved in 0.1 ml of 10 mM tris-HCl buffer-1 mM EDTA (pH 7.0), to obtain a roughly purified product of a plasmid. 1% agarose gel electrophoresis of the product was performed in order to confirm the presence of the plasmid and the molecular size thereof. For comparison, Escherichia coli HB101 strain having a plasmid pBR322 was cultured in LB medium (10 g of Bacto-tryptone, 5g of yeast extract, 10 of NaCl: pH 7.5), and the plasmid therefrom was purified and analyzed in the same manner as above, for comparing the collecting ratio. As a result, presence of the plasmid of Lactobacillus helveticus CP53 strain was confirmed with very high collecting ratio. The plasmid was named pCP53. The collecting amount of pCP53 was approximately 20% of that of the plasmid for Escherichia coli, pBR322 (1 .mu.g/ml medium), which collecting ratio was very high for a Lactobacillus helveticus plasmid.

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L6: Entry 1 of 3 File: USPT Jun 16, 1998

DOCUMENT-IDENTIFIER: US 5766940 A
TITLE: Plasmid and plasmid vector

Brief Summary Text (3):
Lactobacillus helveticus has been used for a long time as a typical dairy lactic acid bacteria starter for production of a fermented milk. It is known that one of the properties of Lactobacillus helveticus is strong proteolytic activity (Yamamoto, N., et al., Biosci. Biotech. Biochem., 58, 776-778 (1994)). Also, it has been reported that the fermented milk by Lactobacillus helveticus contains bioactive peptides which exhibit inhibitory effect against angiotensin converting enzyme which plays an important role in elevating blood pressure (Nakamura, Y. et al., J. Dairy Sci., 78, 777-783 (1995)), and the fermented milk by Lactobacillus helveticus has strong hypotensive effect due to these peptides (Nakamura, Y., et al., J. Dairy Sci., 78, 1253-1257 (1995)). As a method for effective utilization of strains having such properties, methods of genetic engineering utilizing genetic recombination techniques have conventionally been known. Although many plasmids for lactic acid bacteria have been reported, there are few reports on plasmids for lactic acid rod bacteria, Lactobacillus helveticus. Thus, there are few reports on host-vector system of Lactobacillus helveticus .

L6: Entry 2 of 3 File: USPT Dec 9, 1997

DOCUMENT-IDENTIFIER: US 5695796 A
TITLE: Fermented milk product

Brief Summary Text (3):
The lactic acid bacteria of the genus Lactobacillus have long been known as a representative starter for preparation of fermented milk. The lactic acid bacteria of rod shape, such as the genus Lactobacillus produces lactic acid of higher acidity than that of the lactic acid bacteria of coccus such as the genus Lactococcus or the genus Stretococcus, while being frequently higher in its protease activity of decomposing milk protein. In particular, Lactobacillus helveticus exhibits strong protein-decomposing activity. The peptide generated by decomposition of milk protein by its extracellular protease has been reported to exhibit the inhibition activity against angiotensin converting enzyme (Hereinafter referred to as ACE) which is a substance responsible for increase in blood pressure. Similar activity may be noticed with the fermented milk by the Lactobacillus helveticus. These ACE inhibitory peptides have been confirmed to exhibit the activity in lowering the blood pressure with spontaneously hypertensive rats (SHR), as reported by Nakamura, Y. et al NIPPON NOGEI KAGAKU KAISHI, 67, 289, 1993.

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L6: Entry 3 of 3 File: USPT Jul 30, 1996

DOCUMENT-IDENTIFIER: US 5541111 A
TITLE: Lactobacillus helveticus mutants having low increase in acidity of lactic acid during storage

Brief Summary Text (3):
The lactic acid bacteria of the genus Lactobacillus have long been known as a representative starter for preparation of fermented milk. The lactic acid bacteria of rod shape, such as the genus Lactobacillus produces lactic acid of higher acidity than that of the lactic acid bacteria of coccus such as the genus Lactococcus or the genos Stretococcus, while being frequently higher in its protease activity of decomposing milk protein. In particular, Lactobacillus helveticus exhibits strong protein-decomposing activity. The peptide generated by decomposition of milk protein by its extracellular protease has been reported to exhibit the inhibition activity against angiotensin converting enzyme (Hereinafter referred to as ACE) which is a substance responsible for increase in blood pressure. Similar activity may be noticed with the fermented milk by the Lactobacillus helveticus. These ACE inhibitory peptides have been confirmed to exhibit the activity in lowering the blood pressure with spontaneously hypertensive rats (SHR), as reported by Nakamura, Y. et al NIPPON NOGEI KAGAKU KAISHI, 67, 289, 1993.